# EFFECTS OF DIFFERENT WATER SOURCES ON MS2 AND PORCINE ROTAVIRUS REDUCTION IN BIOSAND FILTERS

BY

HANTING WANG

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Environmental Engineering in Civil Engineering
in the Graduate College of the
University of Illinois at Urbana-Champaign, 2015

Urbana, Illinois

Advisers:

Associate Professor Thanh Huong Nguyen
Professor Wen-Tso Liu

## ABSTRACT

Diarrheal diseases caused by pathogens remain a significant cause of death in developing areas, especially for children under the age of five. Biosand filters (BSFs) are a promising technology implemented worldwide that can effectively reduce levels of bacteria and bacteriophages. However, the efficacy of enteric virus reduction in BSFs has not be studied. Furthermore, how the water chemistry of the source water used in BSFs effects virus reduction is not clearly understood. In this study, three bench-scale BSFs were fed daily with groundwater or a cation-free buffered solution to determine MS2 and porcine rotavirus reduction as a function of filter depth, residence time, media ripening, and water source. An integrated cell culture and RT-qPCR assay was developed to quantify rotavirus reduction in water samples collected from the filters. Rotavirus reduction experiments performed in Newmark groundwater reached a cumulative average of 5-$\log_{10}$ (99.999%) reduction after 31 days, exceeding U.S. EPA and World Health Organization standards. Experiments with 1 mM $NaHCO_3$ spiked with MS2 averaged 1.2-$\log_{10}$ reduction after 42 days, and there was not an increasing trend of reduction as a function of depth. Finally, MS2 experiments performed in groundwater reached a cumulative average of 5.36-$\log_{10}$ reduction after 684 days, but reduction did not increase as a function of depth as shown in a previous study. Overall conclusions include that 1) at the same filter age and using the same water source, rotavirus reduction was higher than what was previously seen with MS2, indicating that MS2 is a conservative surrogate for rotavirus, 2) MS2 was reduced to different extents in different water sources, demonstrating that water chemistry, particularly divalent cation concentrations, plays a role on MS2 reduction, and 3) residence time is crucial for increasing virus reduction in all experiments. This is also the first study to determine the efficiency of rotavirus reduction in BSFs,

which is an essential first step in understanding the extent to which BSFs can reduce human enteric viruses, and hence decrease diarrheal disease incidences.

To Mom, Dad, Hanjay, Hanway, Ann, Daniel, and Shelley

## ACKNOWLEDGMENTS

This research was supported by National Science Foundation Graduate Research Program Fellowship (NSF-GRFP), the United States Environmental Protection Agency (U.S. EPA) People, Prosperity, and the Planet (P3) Phase 1 (835515) and the NSF Career grant (0954501) to T.H.N.. I acknowledge my undergraduate research assistants Kazami Brockman, Mingming Li, and Priscila Maradini, who contributed to constructing and operating biosand filters, collecting samples, and quantifying MS2. I also acknowledge Lu Lu and Miyu Fuzawa for collaborating on developing the method for ICC-RT-qPCR. Other experiments, data interpretation, and manuscript preparation were conducted by Hanting Wang under Professor Helen Nguyen's supervision. I would also like to thank my co-advisor Professor Wen-Tso Liu for meaningful discussions about the research, Dr. Shaoying Qi for his support in the lab, and Dr. Rudiger Laufhutte for performing ICP-MS on water samples. Finally, I deeply thank Professor Helen Nguyen for her constant guidance and support throughout this research. Her passion and commitment to global food and water safety have inspired me, and I have learned and grown immensely as a student, researcher, and person while working with her.

## TABLE OF CONTENTS

# CHAPTER 1
# INTRODUCTION

It is estimated that 663 million people worldwide still lack access to improved drinking water sources.[1] There are 4 billion cases of diarrheal diseases worldwide annually caused by consumption and use of unimproved drinking water sources and sanitation facilities.[2] Diarrheal diseases are a leading cause of child morbidity and mortality in impoverished areas, and this remains a significant public health issue.[3] Rotavirus (RV) is the leading cause of diarrhea in the world for children under the age of 5, which led to 450,000 deaths in 2008.[4] It has been shown that household water treatment technologies can help to lower diarrheal disease incidences by 30-40%.[5,6] One of the most promising point-of-use technologies currently available is the biosand filter (BSF), an intermittently-run slow sand filter, which can produce 20-40 L of safe drinking water per day.[5] The filter is easy to implement, use, maintain, is relatively cheap ($12-50/filter), and is sustainable with use up to 12 years.[7,8] 12 L of source water is poured into the top of the filter and flows through the filter with gravity. The filter is designed so that the 12 L of water sits within the pore spaces for a residence time period of 16-32 hours. Another 12 L of water is poured into the filter and pushes the filtered water out of an outlet tube into a storage container.

Previous studies have shown that BSFs can reduce turbidity levels to under 2 NTU and reduce bacteria by >4-$\log_{10}$.[9,10] Many recent studies have focused on reducing virus concentrations, which is more difficult to achieve than reducing bacteria concentrations due to viruses' smaller sizes. These studies observed low and varying levels of MS2 bacteriophage reduction (between 0.37-$\log_{10}$ and 2-$\log_{10}$) in short term experiments of 6-10 weeks long.[9,10,11] However, two recent studies that lasted between 240-300 days showed that MS2 reduction can exceed U.S. EPA and World Health Organization (WHO) standards of 4-$\log_{10}$ reduction.[12,13] One of those studies also showed that after only 4 weeks of experiments using groundwater, MS2 reduction can reach 4-

$log_{10}$, as long as the sand media depth is at least 44 cm long and there is 15.5-31 hours of residence time for the water in the filter.[13] This contrasts with other four week long studies using surface waters which observed low reduction of up to 2-$log_{10}$, even with a 55 cm sand depth and 24 hours of residence time[11] and a 60 cm sand depth and an average of 15.6 hours of residence time.[10]

The reasons for the wide range of MS2 reduction in previous studies are not clear and need to be elucidated to better understand BSF operation and efficacy. Some hypotheses for these differences have been suggested previously. The sand source that was used for the two long-term studies[12,13] was tested for metal oxides (Al, Fe, Mg, Ca, and Zn), which could enhance MS2 adsorption due to positively charged sand surfaces. Using ICP-OES analysis and concentrated hydrofluoric acid solution to digest the sand media, low concentrations of metal oxides were detected,[13] suggesting that it was not the sand source that caused high MS2 reduction in these studies. It has also been hypothesized that sustained filter ripening occurring in two long-term studies[12,13] could have continued to improve MS2 reduction compared to the more typical duration of laboratory BSF experiments of 6-10 weeks.[14] However, one of the studies showed that MS2 reduction can meet 4-$log_{10}$ reduction in 4 weeks,[13] which indicates that other factors are affecting MS2 reduction as well. That study also showed that microbial communities that develop in the BSF play an important role in MS2 reduction.[13]

In addition to microbial communities, the variability in influent water chemistry used in different studies may affect MS2 reduction. However, most of the BSF studies have not considered water chemistry as a factor affecting MS2 reduction and hence, source waters have not been well-characterized. Two studies using Newmark groundwater, which has high divalent cation concentrations (0.6-1.5 mM $Ca^{2+}$ and 1.0-2.3 mM $Mg^{2+}$) saw MS2 reduction reaching 4-$log_{10}$.[12,13] Viruses are uniquely mobile in water,[15] and several previous studies have shown that the

interactions between different viruses and silica surfaces depend on water chemistry. Adsorption of MS2, ΦX174, and human adenovirus are not significantly adsorbed to sand particles when only monovalent cations are present,[16,17] but the presence of $Ca^{2+}$ can enhance adsorption of porcine rotavirus, human adenovirus, and poliovirus to silica surfaces.[15,16] Finally, high divalent cation concentrations have been shown to enhance MS2 adhesion to organic matter coated sand surfaces.[18,19]

These adsorption properties reveal knowledge gaps in BSF research. While divalent cation concentrations influence virus adsorption to sand, a significant limitation to previous MS2 reduction in BSF studies is that only a few different influent water sources have been used, and they are not all well-characterized and are not representative of water sources used in BSFs around the world. Specifically, the water chemistry of water sources has not been systematically characterized and studied, and this is a critical knowledge gap because BSF users treat very different types of water. Another main limitation in current BSF studies is that only bacteria and bacteriophage removal have been studied. There are no studies on enteric virus reduction in BSFs, which is a significant constraint to fully understanding BSF efficacy in environmental conditions because there are physical differences between bacteriophage and enteric viruses.[15] This study addressed the two limitations by evaluating the following as a function of filter depth, media aging, and residence time: MS2 reduction in divalent cation-free water (1 mM $NaHCO_3$) for both unripened and ripened filters, MS2 reduction in Newmark groundwater for a ripened filter of 650-684 days, and porcine rotavirus (PRV) reduction in Newmark groundwater for a ripened filter. This is the first study to determine enteric virus reduction in BSFs, which is an important step to understanding the extent to which BSFs can improve water quality in developing areas. PRV was chosen as the virus to use in this study because it has previously been shown to be more stable and

resistant than human rotavirus to other treatment technologies such as solar inactivation[20]. PRV reduction experiments performed in BSFs showed levels of reduction reaching U.S. EPA and WHO standards. In addition, regardless of the water source used, longer residence times showed higher reduction levels than shorter residence times, demonstrating the importance of residence time on virus reduction that was observed previously.[13] Finally, by comparing MS2 reduction in groundwater and divalent cation-free water, we observed that water source composition plays a critical role in MS2 reduction. Understanding how water chemistry of water sources used in BSFs influences reduction of different viruses can help to enhance future BSF designs to make them more effective.

# CHAPTER 2
# MATERIALS AND METHODS

## 2.1 Cell and Virus Selection

MS2 bacteriophage (ATCC 15597-B1) was obtained from the American Type Culture Collection and was propagated and replicated as described previously.[21] Briefly, *Escherichia coli* (ATCC 15597) was propagated in tryptic soy broth solution, inoculated with MS2, and incubated at 37°C. MS2 was then purified by sequential centrifugation (Eppendorf centrifuge 5416) at 5000 rpm (*g* x 100) for 15 minutes at 20°C, then filtered through a 0.2-µm low-protein-binding polycarbonate track-etched membrane (Whatman Nucleopore, USA), and finally purified and concentrated using polyethylene glycol (PEG) following a previously described protocol.[22] The purified MS2 stock, concentrated to ~$10^{12}$ plaque forming units (PFU) per mL, was stored in 1 mM NaCl at 4°C. Enumeration of MS2 samples was performed using the double agar layer procedure.[23] Dilutions with 30 to 300 plaques were used to calculate the PFU per mL.

Group A porcine rotavirus OSU strain (ATCC VR-892) was propagated in and extracted from MA-104 Clone 1 (ATCC CRL-2378.1) monkey kidney cells, as described elsewhere.[24] Rotavirus (RV) purification was performed following the same protocol as for MS2, except the purified RV stock (~$10^7$ focus forming units (FFU) per mL) was stored in 1 mM NaCl and 0.1 mM $CaCl_2$ solution at 4°C to prevent outer capsid protein denaturation. Enumeration of RV samples were performed using an integrated cell culture and reverse transcription quantitative PCR (ICC-RT-qPCR) method developed, as described in detail below.

## 2.2 Biosand Filter Experiments

Three 4-inch diameter PVC BSFs with a 55 cm sand height were constructed for MS2 and PRV reduction experiments. Seven sampling ports were installed in each filter before packing at the following sand depths (cm): 5.4, 10.9, 16.3, 21.7, 32.6, 43.4, and 54.3. The sand and gravel in the

filters were washed, sieved, and packed according to CAWST recommendations.[25] The upper and lower layers of gravel (1-6 mm and 6-12 mm diameters, respectively) were used to support the sand layer (0.7 mm or smaller diameter). A diffuser plate placed 5 cm above the top of the sand layer reduced the speed of water flow. One of the filters was used in a previous study published by the authors.[13] Tracer tests to verify plug flow in the two newly constructed filers were performed using four or five pore volumes of 0.1 mM NaCl (Figures A1a and A1b).

Feed water for two of the filters was Newmark groundwater (pH 7.7), which has been well characterized in previous studies. One of these filters was used to determine MS2 reduction after 650 days of use, and the other filter was used to determine PRV reduction up to 31 days of use. A third filter was fed with a cation-free buffered solution (1 mM $NaHCO_3$, pH 8.1) for up to 42 days. MS2 and PRV were spiked into the feed waters at concentrations of $10^8$ PFU/mL and $10^6$ FFU/mL, respectively. Maximum filter flow rates were taken during each feed. Short residence time (average of ~10 min) samples were collected once the feed water finished flowing through the filter, and long residence time (20-28 hour) samples were collected immediately prior to another filter feed. All sample volumes were 1 mL, collected in 1.7 mL autoclaved centrifuge tubes, and stored in 4°C until further processing, but no longer than 48 hours after sample collection.

### 2.3 ICC-RT-qPCR for Rotavirus Quantification

#### *2.3.1 Principle of ICC-RT-qPCR*

The two most common methods for quantifying RV are cell culture (focus forming assay or FFU) and RT-qPCR. FFU involves immunostaining MA-104 cells infected by RV through binding of antibodies, an enzyme, and a dye. Stained cells are quantified through counting under a microscope. While this method is well established, it is expensive and has a high detection limit (~$10^2$ FFU/mL). RT-qPCR determines copies of RV present through quantification of cDNA

transcribed from RNA. While RT-qPCR has a lower detection limit (~$10^1$ FFU/mL) than FFU, a weakness of the method is that it detects both infectious and non-infectious RV. For this study, an integrated cell culture and RT-qPCR (ICC-RT-qPCR) method was established to quantify infectious RV. The method produces a calibration curve comparing infectious RV ($\log_{10}$FFU/mL) vs. $\log_{10}$ normalized copy number of replicated RV genomes per cell, which allows for quantification of only infectious RV (Figure A2). This combined method is also more sensitive (detection limit of ~$10^0$ FFU/mL) and faster than the FFU or RT-qPCR methods alone.

Two control tests were performed to test the viability of the method. First, the Newmark groundwater used in this study causes the MA-104 cells to detach when using the FFU method, making it impossible to accurately infect the cells with RV using that method. Hence, the first control test was to ensure that cell detachment would not occur when using the ICC-RT-qPCR method. The second control test was to determine if any components in Newmark groundwater (GW), which was used for biosand filter experiments, inhibit qPCR. cDNA (NSP3 gene for RV) was diluted into two sets, one with nuclease free water (NFW) and one with GW, from the stock solution ($10^{11}$ copies/µL). The dilutions for $10^1$-$10^9$ copies/µL for both sets were used to create calibration curves and to compare the efficiencies and Ct values (Figure A3). Overall, the efficiencies and values for the dilutions with both NFW and GW were similar, and hence it was assumed that components in the groundwater do not inhibit qPCR. The Ct values for both sets of experiments were correlated with the copies number. The slopes of these correlation lines were statistically similar ($p=0.41$). Finally, the efficiencies for the qPCR runs were 98% for NFW and 95% for GW.

*2.3.2 Concentrating Rotavirus in Groundwater Samples*

Prior to infecting confluent MA104 cells, all 16 RV samples collected for each set of data (short residence time samples: influent and Ports 1-7; long residence time samples: effluent and Ports 1-7) from the column were concentrated to increase the detection of RV when performing ICC-RT-qPCR. 0.5 M NaCl (Fisher, Molecular Biology Grade) and 10% weight/volume polyethylene glycol (PEG 6000, Calbiochem, Molecular Biology Grade) were added into each sample tube. The samples were rotated at 15 rpm in 4C for 30 minutes, then centrifuged at 11,500 g for 60 minutes. The supernatant for each sample was pipetted out while being careful not to disrupt the pellets. Finally, 100 µL of 1 mM NaCl + 0.1 mM $CaCl_2$ was added to the centrifuge tubes and the samples were rotated at 15 rpm in 4°C overnight.

*2.3.3 Infecting MA-104 Cells with Rotavirus Samples for ICC-RT-qPCR*

To prepare the sixteen samples collected from the filter for ICC-RT-qPCR, 10 µg/mL trypsin was added to each sample. In addition to these samples, trypsin was also added to a 100 µL sample containing $10^2$ FFU/mL used to spike the feed waters, which was used to determine the concentration of the RV stock at the time of each BSF experiment. A calibration curve of FFU vs. copy numbers determined by RT-qPCR for each experiment was established using four 100 µL samples of the original stock diluted to $10^6$ FFU/mL, $10^4$ FFU/mL, $10^2$ FFU/mL, and $10^0$ FFU/mL. All samples were incubated at 37°C and 5% $CO_2$ for 30 minutes to allow for activation of RV. After this incubation, 100 µL of serum-free Eagle's minimum essential medium (MEM) was added to each sample. This gave a total volume of 200 µL per sample for a total of 21 samples per set of data.

21 wells out of a 24 well plate of confluent MA-104 cells were rinsed twice with 500 µL phosphate buffered saline (PBS) per well to remove unbound cells. 150 µL of serum-free MEM +

RV + trypsin solution was added to each of the 21 wells. The samples were incubated at 37°C for 30 minutes to allow RV binding and penetration. The cells were then rinsed twice with 500 µL serum-free MEM per well to remove unbound viruses. 500 µL of serum-free MEM was added into each well and the samples were incubated at 37°C and 5% $CO_2$ for 18 hours.

*2.3.4 Collection of MA-104 Cells for GAPDH Gene Quantification*

After the 18 hours of incubation, one well in the 24 well plate of confluent MA-104 cells was used to create a calibration curve to determine the total number of cells exposed to the infectious RV (Figure A4). This well was rinsed twice with 500 µL serum-free MEM to remove unbound cells. 200 µL of 0.25% EDTA-trypsin was added to the well and the cells detached while incubating for five minutes in 37°C and 5% $CO_2$. After detachment, 500 µL of serum-free MEM was added to the cells and was pipetted up and down to mix sufficiently. 10 µL of this solution was pipetted onto a hemocytometer to count the number of cells in the solution. Another 150 µL of cells + serum-free MEM was collected for RNA extraction.

*2.3.5 Extracting RNA from Cells Infected with Rotavirus*

Prior to extraction, a PCR hood specifically designated for RNA extraction was wiped down with 70% ethanol, UV'ed for 10 minutes, and wiped down with RNase away. 350 µL of TKR lysis buffer from E.Z.N.A Total RNA I (Omega Bio-Tek) extraction kit was added into each well, which lysed and detached the cells from the bottom of the wells. RNA was extracted from the virus-infected cells following the manufacturer's instructions. Total RNA for each sample was eluted with 40 µL of nuclease-free water and samples were stored at -80°C until further processing. All samples were only freeze-thawed one time to prevent instability of RNA. The extracted RNA from the MA104 cells was subjected to qPCR to track the GAPDH housekeeping gene.

*2.3.6 Quantification of NSP3 and GAPDH Gene in Rotavirus Samples*

RV quantification through ICC-RT-qPCR presented as FFU/mL was normalized by the number of MA-104 cells per well. In order to determine the RV stock concentration, a calibration curve was prepared by making ten-fold dilutions of a plasmid cDNA standard (2207 bp) of $10^{12}$ copies/µL containing the RV NSP3 gene (212 bp) to concentrations between $10^3$ and $10^8$ copies/µL (Figure A5). These concentrations were used as the linear range of quantifiable copy numbers established by the calibration curve. Ct values obtained from the biosand filter samples also fell within the standard concentration range. The primers that were ordered from Integrated DNA Technologies and used to quantify RV through RT-qPCR were JVKF (5'-CAGTGGTTGATGCTCAAGATGGA-3') and JVKR (5'-TCATTGTAATCATATTGAATACCCA-3').[26]

Because the number of MA-104 cells per well cannot be assumed to be the same in each well, in order to accurately quantify the amount of RV infecting the MA-104 cells per well, the number of cells per well needed to be quantified as well. To do this, the GAPDH housekeeping gene was tracked through qPCR based on the fact that there are two GAPDH housekeeping genes per cell.[27] The GAPDH primers that were ordered from Integrated DNA Technologies and used for RT-qPCR were GAPDHF (5′-AATCCCATCACCATCTTCCAG-3′) and GAPDHR (5′-AAATGAGCCCCAGCCTTC-3′).[28] Using the cell counts from the hemocytometer as a basis of the cell concentration per well (~$10^7$ copies/µL), a calibration curve to quantify the GAPDH gene was made consisting of ten-fold dilutions (concentrations between $10^3$ and $10^7$ copies/µL) of the extracted RNA from the cells-only well (Figure A4). For each sample, the NSP3 and GAPDH genes were quantified in the same thermal cycle. Dilutions of the four primers and cDNA standard were stored in -20°C and were thawed on ice prior to each RT-qPCR run.

*2.3.7 Calibration Curve for Quantification of Infectious Rotavirus*

Using the GAPDH and JVK primer calibration curves (Figures A4 and A5) and the FFU concentrations of the RV stock, a calibration curve comparing infectious RV in log (FFU/mL) vs. normalized log copy number of replicated RV genomes per cell is produced for each experiment (Figure A2). This calibration curve is powerful because it can be used to quantify only infectious RV, which is what is important to quantify for BSF virus reduction experiments. By determining the normalized log copy number of replicated RV genomes per cell for each of the 16 samples collected per experiment, one can use the calibration curve (Figure A2) to determine the log(FFU/mL) of each sample. Using the concentration of the influent as a base line, log reduction can be calculated for the rest of the samples.

*2.3.8 RT-qPCR Protocol*

RT-qPCR was performed in 384 well optical reaction plate using a Bio-Rad iTaq Universal SYBR Green One-Step Kit and an Applied Biosystems 7900HT Fast Real-Time PCR System (Carl R. Woese Institute for Genomic Biology, Urbana, IL 61801). Each reaction consisted of 10 µL total volume: 7 µL of RT-qPCR mastermix and 3 µL of RNA template. Two mastermix solutions were made, one for the NSP3 gene and one for the GAPDH gene. The solutions consisted of a mixture of 1X iTaq universal SYBR green reaction mix, 1X iScript reverse transcriptase, nuclease-free water, and 300 nM each forward (JVKF) and reverse (JVKR) primers for RV and 178 nM each forward (GAPDHF) and reverse (GAPDHR) primers for GAPDH. The same thermal cycling conditions used for both primer sets was follows: 10 min reverse transcription step at 48°C; 1 min denaturation and iTaq activation step at 95°C; 35 cycles of denaturing at 95°C for 15 s, annealing at 54°C for 20 s, and extension at 60°C for 30 s. A dissociation cycle of 15 s at 95°C, 15 s at 54°C, and 95°C for 15 s was performed to assess for nonspecific amplification. Each RNA sample was

run in triplicates and the average Ct value between the triplicates was used to calculate copy numbers. A negative control was included in every RT-qPCR run using the nuclease-free water used to make the mastermix solutions.

The ranges of efficiencies were 86-101% and 88-118% for the JVK and GAPDH calibration curves, respectively. The detection limit for a Ct value of 35 was between 20 copies/µL and 80 copies/µL for JVK and GAPDH primers, respectively. The $R^2$ values of the calibration curves were consistently between 0.98-0.99 for both JVK and GAPDH primers.

**2.4 Data Analysis**

Ct values were obtained in triplicates for all samples: influent, effluent, ports, cDNA calibration curve, cell calibration curve, RV stock calibration curve, and negative control. The average Ct value between the triplicates was used for quantification of NSP3 and GAPDH genes.

# CHAPTER 3
# RESULTS AND DISCUSSION

## 3.1 MS2 Reduction Experiments Using 1 mM $NaHCO_3$ as the Source Water

In order to determine if high divalent cation concentrations (0.6-1.5 mM $Ca^{2+}$ and 1.0-2.3 mM $Mg^{2+}$) in the influent water source affects MS2 reduction, as hypothesized in a previous study,[13] MS2 reduction experiments as a function of depth and residence time were performed using 1 mM $NaHCO_3$. 1 mM $NaHCO_3$ was used for three reasons: 1) the pH of 1 mM $NaHCO_3$ (pH 8.1) is similar to the pH of Newmark groundwater (pH 7.7), 2) MS2 is stable and does not aggregate in 1 mM $NaHCO_3$,[19] and 3) there are no divalent cations in 1 mM $NaHCO_3$.

Figure 1 shows the reduction of MS2 in 1 mM $NaHCO_3$ as a function of filter depth from Days 11-42, which encompasses an unripened filter (up to 28 days)[13] and the start of the ripened period for the filter. Samples were taken from seven ports for both short residence time (SRT) of ~10 min and long residence time (LRT) of 20-25 hours. MS2 reduction reached a cumulative average of 0.35-$\log_{10}$ for SRT and 1.2-$\log_{10}$ for LRT. MS2 reduction is not statistically different between all ports for both short and long residence times ($p>0.09$ and $p>0.33$ for all ports for SRT and LRT, respectively), meaning there is no increase or decrease in trend as a function of depth. This contrasts previously published data that determined MS2 reduction as a function of depth and residence time using Newmark groundwater in another PVC filter identical to the one used in these experiments.[13] In the same period of time (prior to filter ripening), the average cumulative MS2 reduction in the groundwater column was 1.5-$\log_{10}$ higher than in the $NaHCO_3$ column for SRT and 3.5-$\log_{10}$ higher for LRT. Furthermore, there was an exponential dependence of MS2 reduction on depth throughout the groundwater column.[13]

In another study, MS2 reduction in Cane Creek Reservoir water was observed as a function of depth at 10 cm and 30 cm in a filter that was run between 42-52 days.[29] This study showed low

reduction (~1-$\log_{10}$) at both 10 cm and 30 cm depths, and the reductions were not significantly different between the two depths. Although the creek water used in the study was not characterized, it can be assumed that since the water is surface water, it is relatively soft compared to groundwater,[30] which can explain why the reduction levels are low and not significantly different at different depths of the filter. Similarly, in another study using Lac St Louis water in Quebec, MS2 reduction observed as a function of depth showed reduction averaging between 1-$\log_{10}$ and 2-$\log_{10}$ throughout the filter.[11] With high variability of reduction observed between samples, it was only concluded that more reduction occurred at 10 cm and 30 cm compared to 5 cm and 55 cm. It is difficult to say if the reductions were significantly different at different depths, but overall, the reduction levels were lower than seen when using groundwater.[13]

These varying levels of MS2 reduction using different water sources suggest that different concentrations of divalent cations in water sources affect MS2 reduction efficacy. In particular, the hypothesis that high divalent cation concentrations in the source water affects MS2 reduction was proven. It has been suggested previously that divalent cations cause charge neutralization of MS2,[31] which has an isoelectric point of 3.6.[21] Quartz sand packed in BSFs has an isoelectric point of 2.44,[32] indicating that there is repulsion between MS2 and sand because they are both negatively charged in environmental conditions. However, if divalent cations are present and charge neutralization of MS2 occurs, there will be less repulsion between MS2 and sand, which means that less MS2 reduction would be observed in 1 mM $NaHCO_3$ and surface waters compared to waters with divalent cations present. Finally, it is important to note that although the MS2 reduction for SRT is negligible, observing over 1-$\log_{10}$ reduction with 20-25 hours of residence time shows the significance of physical reduction that occurs in the filter with sufficient residence time, similar to what was shown in a previous study.[13]

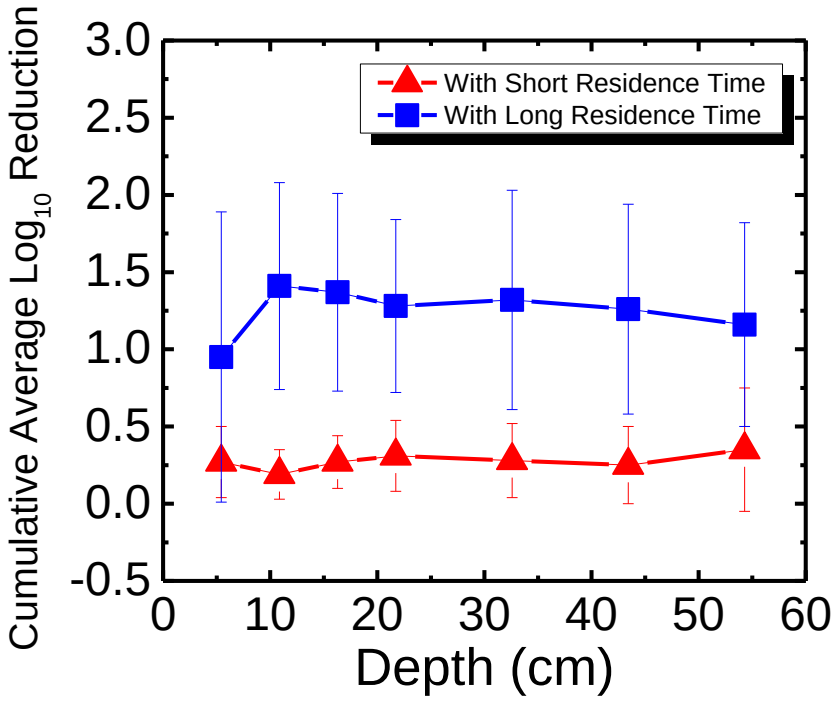


**Figure 1.** Cumulative average $\log_{10}$ reduction of MS2 in 1 mM $NaHCO_3$ as a function of filter depth and short residence time (~10 min) and long residence time (20-25 hours) for Days 11-42.

### 3.2 Long-Term MS2 Reduction in Newmark Groundwater

Studies have shown that BSFs have sustainable use in the field up to 12 years.[7] While it is encouraging to see that people continue to use their filters, no studies have determined pathogen reduction efficacy in a filter that has been used for more than 300 days. It is important to determine how pathogen reduction efficacy changes with time to better understand the mechanism of pathogen reduction in the filter. This is the first study to determine the efficacy and trend of MS2 reduction for a biosand filter that has been run for more than 650 days. Figure 2 shows MS2 reduction in Newmark groundwater as a function of filter depth from Days 650-684. Samples were taken from seven ports for both short residence time (~10 min) and long residence time (24-29 hours). MS2 reduction reached a cumulative average of 0.52-$\log_{10}$ for SRT and 5.36-$\log_{10}$ for LRT. MS2 reduction was only statistically different between Ports 2 and 3 and 2 and 7 for SRT ($p<0.03$), and Ports 2 and 7, 3 and 7, and 4 and 7 for LRT ($p<0.03$).

This is the same PVC port filter that was used in a previous study[13] for MS2 reduction experiments for Days 9-28, which had a shorter SRT (10 min) compared to a full-scale BSF (45 min). Also reported in the study was the concrete port filter that ran for 240 days and showed an average cumulative MS2 reduction of 5.16-$\log_{10}$ for SRT and 5.64-$\log_{10}$ for LRT.[13] Although both the PVC port filter used in this study and the concrete port filter used in the previous study[13] were fully ripened, there was significantly lower MS2 reduction observed for SRT for the PVC port filter. This could suggest that a SRT period of 10 min was not enough time for significant MS2 reduction to occur when the filter has ripened, even when using water that had high divalent cations. In another study that used Lac St Louis, similar reduction levels (~0.5-$\log_{10}$) were observed at different depths throughout a 10 cm diameter acrylic filter on Day 60 with residence

time of 4 hours, but reduction levels reached around 2-$\log_{10}$ with 24 hours of residence time,[11] once again showing how residence time can increase reduction regardless of the water source used.

Furthermore, for the concrete port filter used in a previous study,[13] there was increasing cumulative MS2 reduction as a function of depth for both SRT and LRT, where the most significant reduction occurred in the first 5 cm of the filter. However, as shown in Figure 2, for the PVC port filter, there was no increasing or decreasing trend for both SRT and LRT ($p>0.26$ for all ports for SRT except between Ports 2-3 where $p = 0.03$, and $p>0.05$ for all ports for LRT). One possible reason why MS2 reduction did not increase with depth could be because of a shift in microbial community structure throughout the filter. Previously, it was shown that the highest abundance and diversity of species populated the first 5 cm of the filter, allowing for the most reduction in the biolayer.[13] Perhaps with time (>650 days), the microbial communities shifted and evened out throughout the filter. In order to determine if the hypothesis is correct, taking core sand samples from this filter and performing sequencing techniques to determine microbial communities at different depths would be necessary. While this was not done within this study, future work should focus on understanding further how microbial communities influence long-term virus reduction, which is important for communities where BSFs are the main technology for drinking water treatment. In the meantime, since this is the only study to determine MS2 reduction for more than 300 days and that MS2 reduction can reach U.S. EPA and WHO standards only with long residence times, BSF users should operate the filters with the typical 16-32 hours of residence time[13] to ensure that more effective virus reduction takes place.

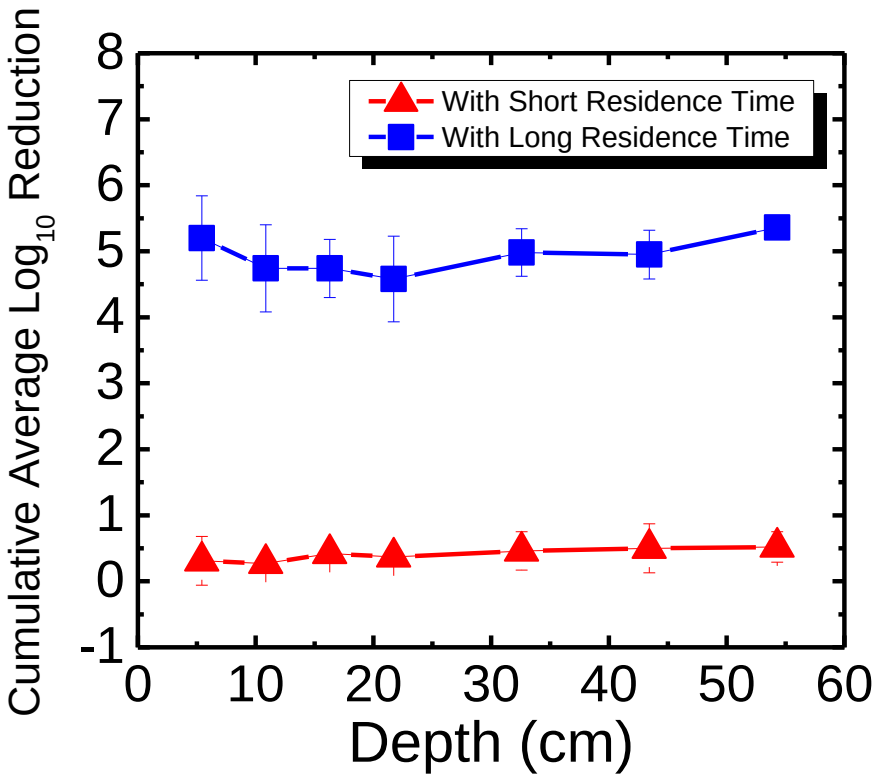


**Figure 2.** Cumulative average $\log_{10}$ reduction of MS2 in Newmark groundwater as a function of filter depth and short residence time (~10 min) and long residence time (24-29 hours) for Days 650-684.

### 3.3 Porcine Rotavirus Reduction in Newmark Groundwater

Another limitation to published studies on BSFs is that only bacteria and bacteriophage reduction efficacy has been studied. The lack of studies on enteric virus reduction in BSFs inhibits fully understanding how well the filter works in field conditions. This is the first study to determine enteric virus reduction in a BSF. PRV was spiked in Newmark groundwater and reduction was observed as a function of depth and residence time. Figure 3 shows PRV reduction in Newmark groundwater as a function of filter depth from Days 1-31. Samples were taken from seven ports for both SRT (~10 min) and LRT (24 hours). PRV reduction reached a cumulative average of 3.54-$\log_{10}$ for SRT and 4.92-$\log_{10}$ for LRT, which meets U.S. EPA and WHO standards for virus reduction. Similar to the MS2 reduction shown in Newmark groundwater for the PVC port column in a previous study,[13] PRV reduction followed an exponential increase of reduction as a function of depth for SRT ($R^2$=0.97). Although there was not an exponential increase of reduction as a function of depth for LRT ($R^2$=0.40), there was still significantly higher reduction seen during LRT than SRT. This indicates that residence time also improves PRV reduction due to more contact between the water and sand.

Even without ripening, PRV reduction reached 3-$\log_{10}$ in the first 5 cm of the filter (Port 1) alone for LRT, which is 1-$\log_{10}$ more than what was seen in MS2 reduction in Port 1 for LRT.[13] The extent of PRV reduction in Port 1 is similar reduction to the cumulative average MS2 reduction in groundwater after 240 days for Port 1. This suggests that high levels of RV reduction may not be entirely dependent on filter ripening, as shown in a previous study for MS2,[13] but could occur for certain viruses and filter usage conditions. For example, it has been shown previously that RV aggregates in high concentrations of divalent cations.[15] If water sources with particular water

chemistry (e.g. with divalent cations) were used, a 55 cm filter may not be necessary if the water has enough residence time in the filter.

Similar to how this is the only study determining enteric virus reduction in BSFs, there are also limited studies looking at enteric virus reduction in slow sand filtration. Although the results from the studies vary, important conclusions can be drawn. In one study, reovirus was removal by at least 4-$\log_{10}$ after 7, 91, and 147 days when using river water as a water source in a 15 cm diameter filter with a 90 cm sand depth and 8 hours of residence time.[33] The study also showed an insignificant difference between removal between ripened and unripened filters. The long filter depth could have played a role in the high removal even before ripening, especially since it was shown that the majority of the removal took place within the top 42 cm of the filter. In another study, poliovirus removal varied between 0.1-$\log_{10}$ and 4-$\log_{10}$ depending on water source, sand type, and flow rate.[34]

Through this current study and previous studies, it can be concluded that certain filter characteristics such as water source and filter depth greatly influence enteric virus reduction efficacy. Because there has been a wide variety of virus reduction efficacies seen under different filter conditions, more studies need to be done with different viruses and different water sources to get a more complete picture of virus reduction efficacy in BSFs. This is the only study on RV reduction in BSFs, and since RV is the leading cause of diarrheal diseases among children under the age of 5 around the world, it is recommended that filter users use the CAWST Version 10 BSF, as they have been shown in this study to reduce RV up to U.S. EPA and WHO standards.

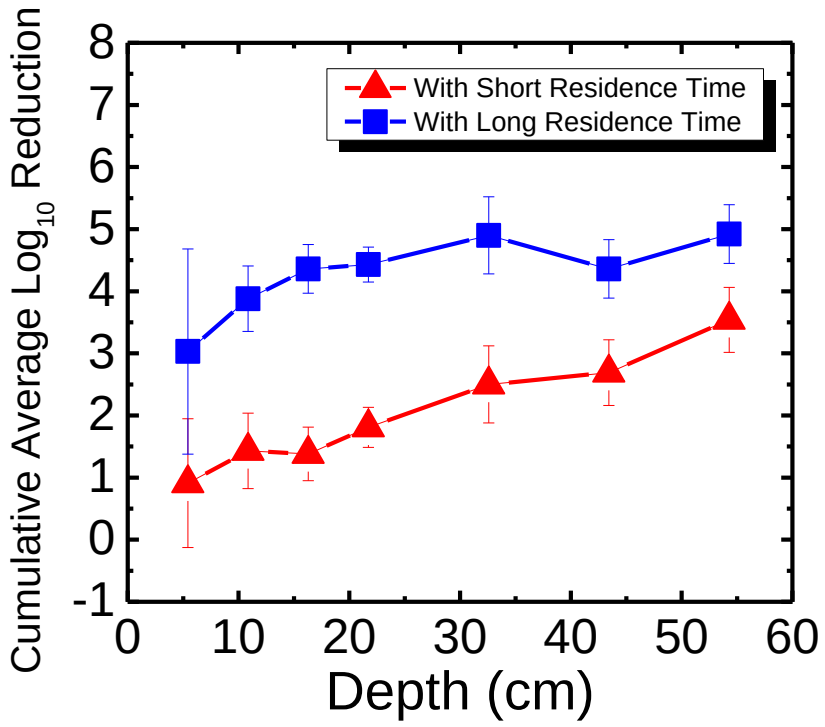


**Figure 3.** Cumulative average $\log_{10}$ reduction of PRV in Newmark groundwater as a function of filter depth and short residence time (~10 min) and long residence time (24 hours) for Days 1-31.

# CHAPTER 4
# CONCLUSIONS

Overall, this study shed light on two major limitations in previous BSF studies. First, it was shown that the water chemistry of the influent source waters affects MS2 reduction efficacy, and this influence of water chemistry differs between unripened and ripened periods. While MS2 reduction using Newmark groundwater that has high divalent cation concentrations exceeded U.S. EPA and WHO standards of 4-$\log_{10}$ reduction in a previous study,[13] MS2 reduction using 1 mM $NaHCO_3$, which has no cations, showed low reduction (1.2-$\log_{10}$). Second, this was the first study to determine the efficacy of enteric virus reduction in BSFs. After 31 days, PRV reduction reached 4.92-$\log_{10}$, which exceeded U.S. EPA and WHO standards. This level of reduction was seen when using Newmark groundwater as the source water, and is comparable to previous studies looking at reovirus and poliovirus removal in slow sand filtration. In addition, an ICC-RT-qPCR method was developed to more efficiently and effectively quantify PRV reduction in the BSF samples.

Due to time constraints, some experiments that would more comprehensibly address the two limitations mentioned above were not completed. Future work should focus on these experiments. First, RV reduction efficacy was only studied for the BSF's unripened period. While this is invaluable data, showing that enteric viruses can be reduced effectively by BSFs, long-term studies of RV and short- and long -term studies of other enteric viruses are needed to fully understand the efficacy of enteric virus reduction in BSFs. Furthermore, these experiments should explore different water sources, especially those that mimic field conditions, and water chemistry should be systematically characterized. Only two water sources have been characterized in BSF studies[13] and a few water sources have been characterized for slow sand filtration.[33,34] While it is clear that water chemistry plays a role on virus reduction, there is still much uncertainty about how water chemistry affect virus reduction. Finally, microbial community analysis of the filters, similar

to what was done for the first time on one BSF in a previous study,[13] should be performed on more filters with different water sources to better understand the mechanism of virus reduction in the filter. Results from these experiments would help to improve the BSF and quality of life of BSF users.

## REFERENCES


1. WHO/UNICEF Joint Monitoring Programme for Water Supply and Sanitation. Progress on Sanitation and Drinking-Water: 2015 Update and MDG Assessment. 90 (2015).
2. WHO/UNICEF. Global Water Supply and Sanitation Assessment: 2000 Report. 87 (2000). doi:http://www.who.int/water_sanitation_health/monitoring/globalassess/en/
3. World Health Organization (WHO). Diarrhoeal disease. at <http://www.who.int/mediacentre/factsheets/fs330/en/>
4. World Health Organization (WHO). Estimated rotavirus deaths for children under 5 years of age: 2008, 453000. at <http://www.who.int/immunization/monitoring_surveillance/burden/estimates/rotavirus/en/>
5. Sobsey, M. D., Stauber, C. E., Casanova, L. M., Brown, J. M. & Elliott, M. a. Point of use household drinking water filtration: A practical, effective solution for providing sustained access to safe drinking water in the developing world. *Environ. Sci. Technol.* **42,** 4261–4267 (2008).
6. Fewtrell, L. *et al.* Water, sanitation, and hygiene interventions to reduce diarrhoea in less developed countries: a systematic review and meta-analysis. *Lancet. Infect. Dis.* **5,** 42–52 (2005).
7. Sisson, A. J., Wampler, P. J., Rediske, R. R. & Molla, A. R. An assessment of long-term biosand filter use and sustainability in the Artibonite Valley near Deschapelles, Haiti. *J. Water, Sanit. Hyg. Dev.* **3,** 51–60 (2013).
8. Centre for Affordable Water and Sanitation Technology (CAWST). Biosand Filters Knowledge Base. Fact Sheet: Biosand Filter. at <http://biosandfilters.info/technical/fact-sheet-biosand-filter>
9. Elliott, M. a, Stauber, C. E., Koksal, F., DiGiano, F. a & Sobsey, M. D. Reductions of E. coli, echovirus type 12 and bacteriophages in an intermittently operated household-scale slow sand filter. *Water Res.* **42,** 2662–70 (2008).
10. Jenkins, M. W., Tiwari, S. K. & Darby, J. Bacterial, viral and turbidity removal by intermittent slow sand filtration for household use in developing countries: experimental investigation and modeling. *Water Res.* **45,** 6227–39 (2011).
11. Young-Rojanschi, C. & Madramootoo, C. Intermittent versus continuous operation of biosand filters. *Water Res.* **49,** 1–10 (2014).
12. Bradley, I., Straub, A., Maraccini, P., Markazi, S. & Nguyen, T. H. Iron oxide amended biosand filters for virus removal. *Water Res.* **45,** 4501–10 (2011).
13. Wang, H. *et al.* MS2 bacteriophage reduction and microbial communities in biosand filters. *Environ. Sci. Technol.* **48,** 6702–6709 (2014).
14. Elliott, M., Stauber, C., DiGiano, F., de Aceituno, A. & Sobsey, M. Investigation of E. coli and Virus Reductions Using Replicate, Bench-Scale Biosand Filter Columns and Two Filter Media. *Int. J. Environ. Res. Public Health* **12,** 10276–10299 (2015).
15. Gutierrez, L., Mylon, S. E., Nash, B. & Nguyen, T. H. Deposition and aggregation kinetics of rotavirus in divalent cation solutions. *Environ. Sci. Technol.* **44,** 4552–7 (2010).
16. Wong, K., Mukherjee, B., Kahler, A. M., Zepp, R. & Molina, M. Influence of inorganic ions on aggregation and adsorption behaviors of human adenovirus. *Environ. Sci. Technol.* **46,** 11145–11153 (2012).
17. Jin, Y., Yates, M. V, Thompson, S. S. & Jury, W. A. Sorption of viruses during flow

through saturated sand columns. *Environ. Sci. Technol.* **31,** 548–555 (1997).
18. Pham, M., Mintz, E. a. & Nguyen, T. H. Deposition kinetics of bacteriophage MS2 to natural organic matter: Role of divalent cations. *J. Colloid Interface Sci.* **338,** 1–9 (2009).
19. Yuan, B., Pham, M. & Nguyen, T. H. Deposition kinetics of bacteriophage MS2 on a silica surface coated with natural organic matter in a radial stagnation point flow cell. *Environ. Sci. Technol.* **42,** 7628–7633 (2008).
20. Romero-Maraccini, O. C. *et al.* Sunlight-induced inactivation of human Wa and porcine OSU rotaviruses in the presence of exogenous photosensitizers. *Environ. Sci. Technol.* **47,** 11004–11012 (2013).
21. Gutierrez, L. *et al.* Adsorption of rotavirus and bacteriophage MS2 using glass fiber coated with hematite nanoparticles. *Water Res.* **43,** 5198–208 (2009).
22. Nguyen, T. H. *et al.* The RNA core weakly influences the interactions of the bacteriophage MS2 at key environmental interfaces. *Soft Matter* **7,** 10449 (2011).
23. Adams, M. *Bacteriophages*. (Interscience Publishers, 1959). at <https://archive.org/stream/bacteriophages00adam#page/n5/mode/2up>
24. Rolsma, M. D., Gelberg, H. B. & Kuhlenschmidt, M. S. Assay for evaluation of rotavirus-cell interactions: identification of an enterocyte ganglioside fraction that mediates group A porcine rotavirus recognition. *J. Virol.* **68,** 258–68 (1994).
25. CAWST. *Biosand filter manual design, construction, installation, operation and maintenance*. (2009).
26. Jothikumar, N., Kang, G. & Hill, V. R. Broadly reactive TaqMan® assay for real-time RT-PCR detection of rotavirus in clinical and environmental samples. *J. Virol. Methods* **155,** 126–131 (2009).
27. Seidler, N. W. *GAPDH: Biological Properties and Diversity*. (Springer, 2012).
28. Bhowmick, R., Halder, U. C., Chattopadhyay, S., Nayak, M. K. & Chawla-Sarkar, M. Rotavirus-encoded nonstructural protein 1 modulates cellular apoptotic machinery by targeting tumor suppressor protein p53. *J. Virol.* **87,** 6840–6850 (2013).
29. Elliott, M. a, Digiano, F. a & Sobsey, M. D. Virus attenuation by microbial mechanisms during the idle time of a household slow sand filter. *Water Res.* **45,** 4092–4102 (2011).
30. Crittenden, J. C.; Trussell, R. R.; Hand, D. W.; Howe, K. J.; Tchobanoglous, G. *MWH's Water Treatment: Principles and Design*. (John Wiley & Sons, Inc., 2012).
31. Mylon, S. E. *et al.* Influence of salts and natural organic matter on the stability of bacteriophage MS2. *Langmuir* **26,** 1035–1042 (2010).
32. Jada, A., Ait Akbour, R. & Douch, J. Surface charge and adsorption from water onto quartz sand of humic acid. *Chemosphere* **64,** 1287–95 (2006).
33. McConnell, L. K., Sims, R. C. & Barnett, B. B. Reovirus removal and inactivation by slow-rate sand filtration. *Appl. Environ. Microbiol.* **48,** 818–825 (1984).
34. Robeck, G. G., Clark, N. E. & Dostal, K. A. Effectiveness of water treatment processes in virus removal. *J. Awwa* **54,** 198A–205A (1962).

## APPENDIX A: SUPPLEMENTARY FIGURES

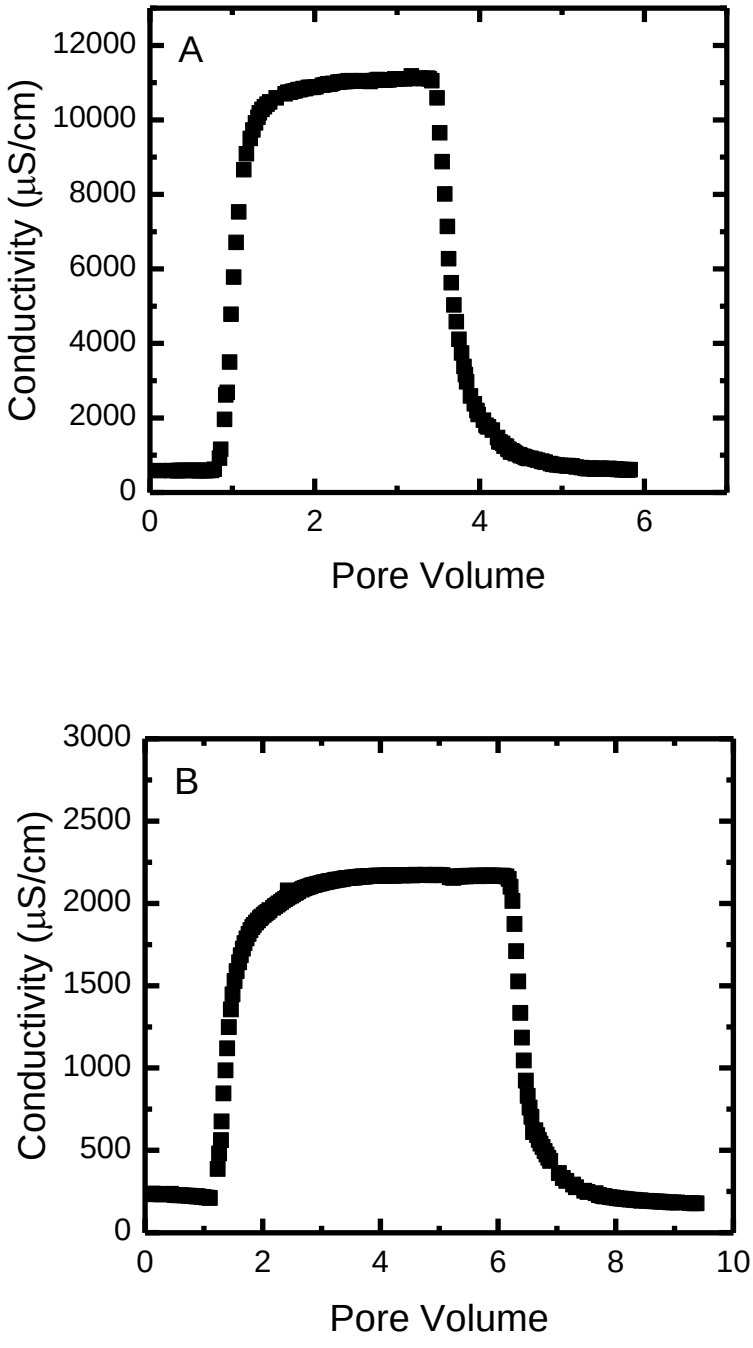


**Figure A1a.** Tracer test curve for biosand filter fed with 1 mM $NaHCO_3$. **Figure A1b.** Tracer test curve for biosand filter fed with Newmark groundwater.

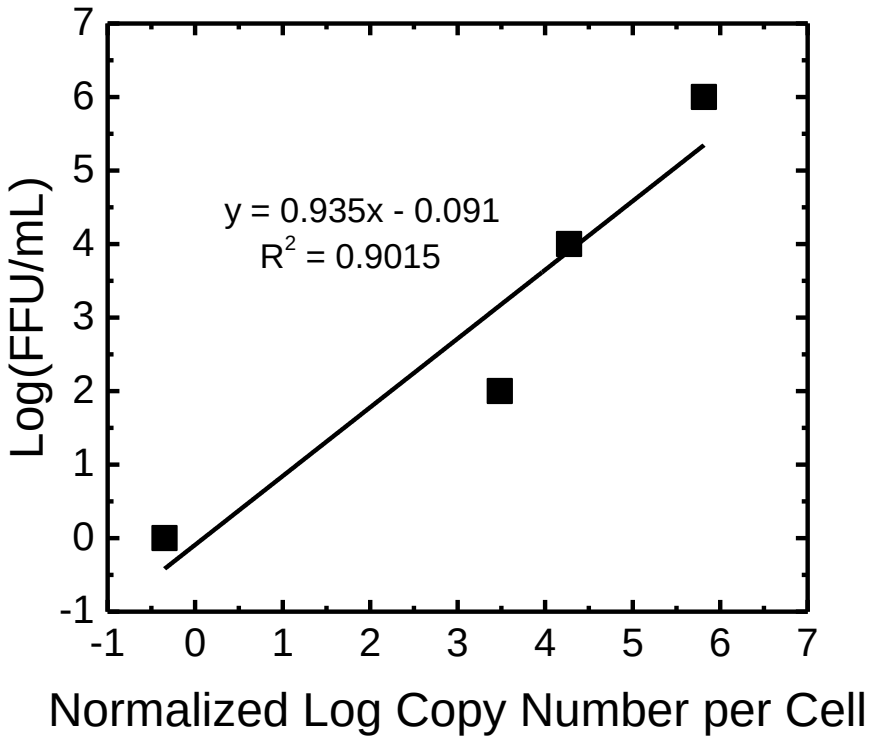


**Figure A2.** Calibration curve comparing infectious RV in log (FFU/mL) vs. normalized log copy number of replicated RV genomes per cell, which allows for quantification of only infectious RV.

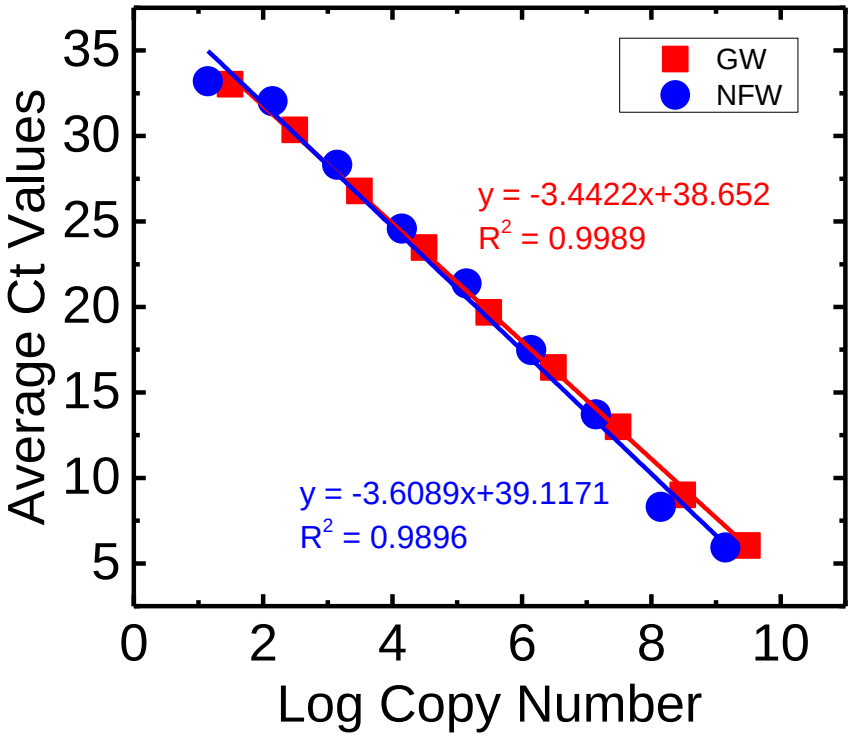


**Figure A3.** Calibration curves comparing cDNA for the NSP3 gene in nuclease-free water and in Newmark groundwater.

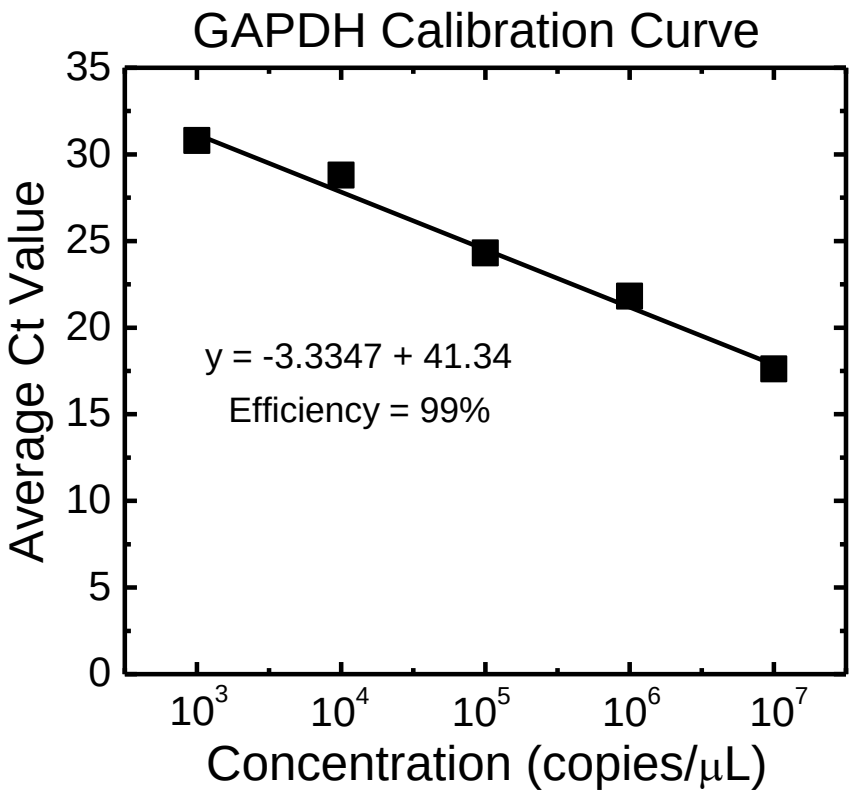


**Figure A4.** Calibration curve for the GAPDH housekeeping gene at concentrations from $10^3$-$10^7$ copies/μL for one experiment.

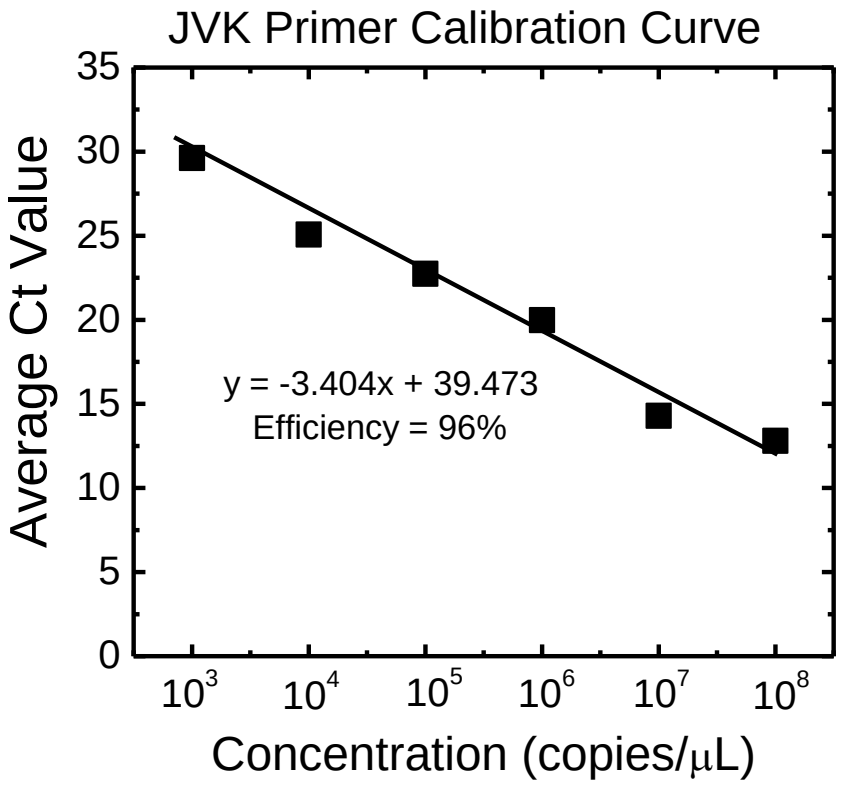


**Figure A5.** Calibration curve for the plasmid cDNA standard for the RV NSP3 gene at concentrations from $10^3$-$10^8$ copies/μL for one experiment.